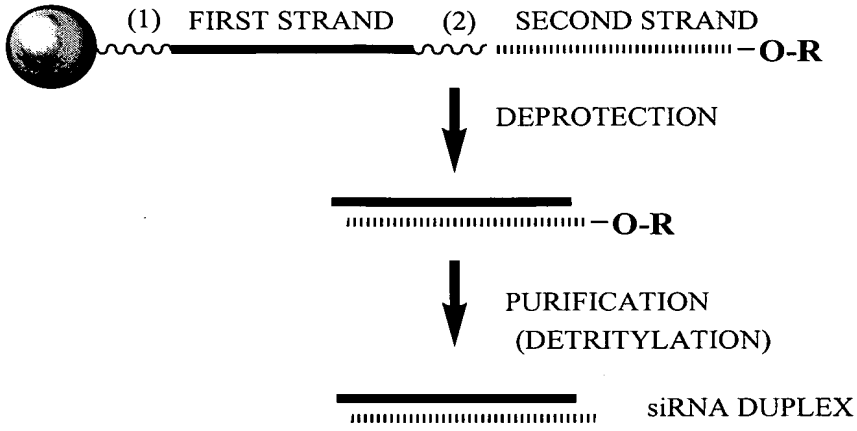
Figure 1

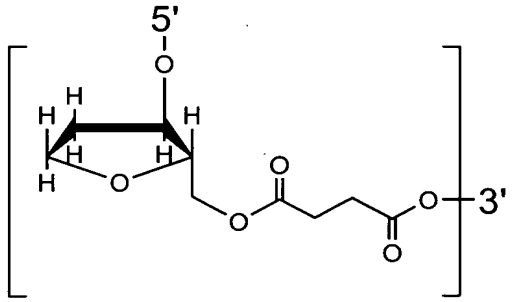


= SOLID SUPPORT

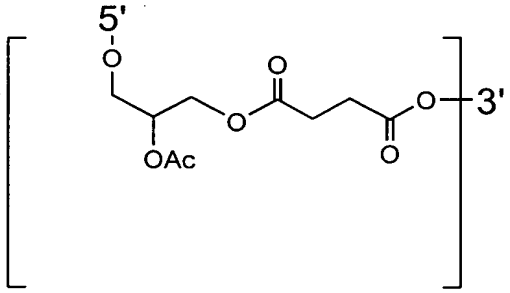
R = TERMINAL PROTECTING GROUP
FOR EXAMPLE:
DIMETHOXYTRITYL (DMT)

(1) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)

(2) = CLEAVABLE LINKER
(FOR EXAMPLE: NUCLEOTIDE SUCCINATE OR INVERTED DEOXYABASIC SUCCINATE)



INVERTED DEOXYABASIC SUCCINATE LINKAGE



GLYCERYL SUCCINATE LINKAGE

Figure 2

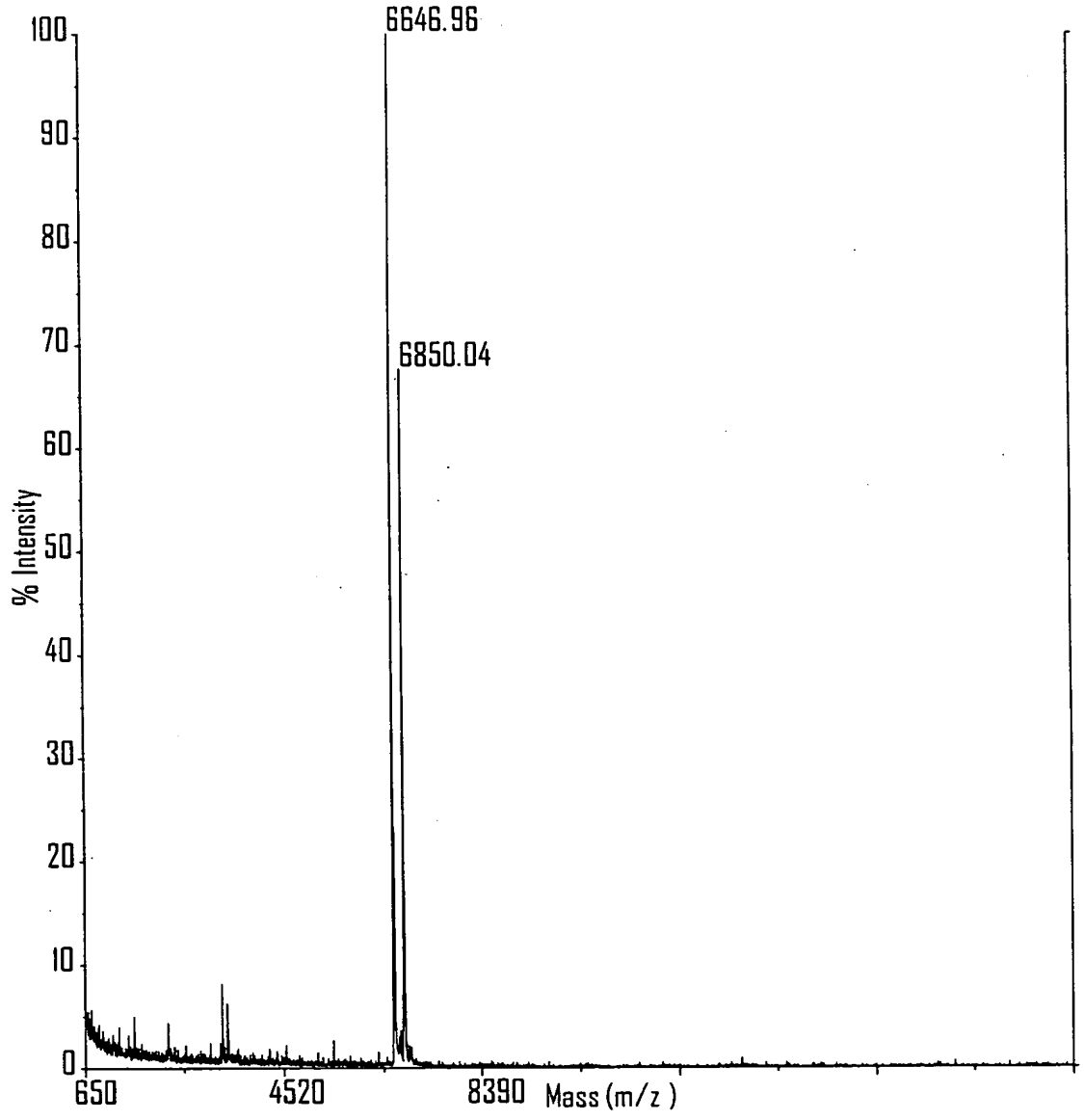

6646.96
6850.04
100
90
80
70
60
50
40
30
20
10
0
% Intensity
650
4520
8390
Mass (m/z)

Figure 3

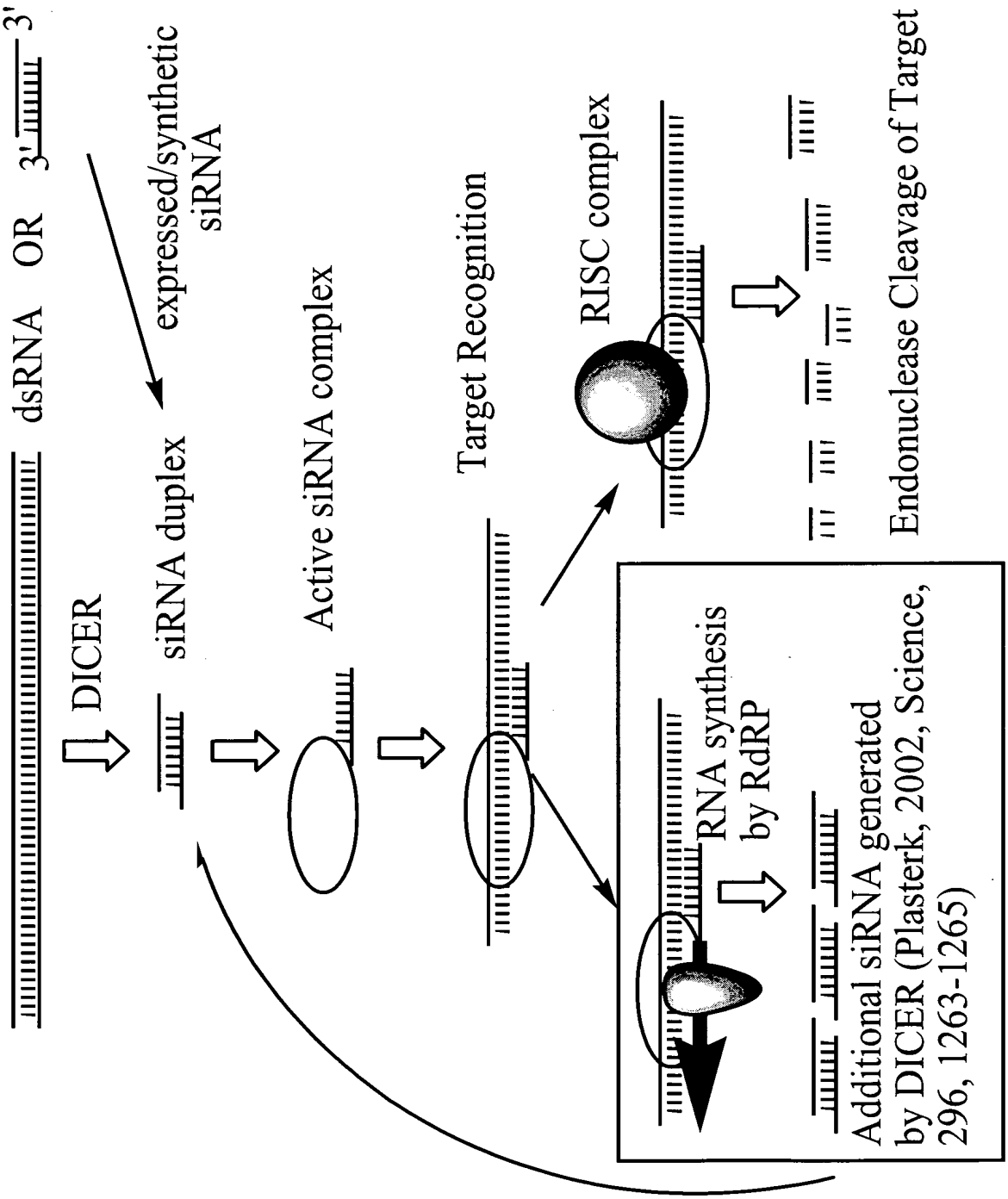

dsRNA OR 3' 3'
DICER
siRNA duplex
expressed/synthetic siRNA
Active siRNA complex
Target Recognition
RISC complex
RNA synthesis by RdRP
Endonuclease Cleavage of Target
Additional siRNA generated by DICER (Plasterk, 2002, Science, 296, 1263-1265)

Figure 4

A

SENSE STRAND (SEQ ID NO 55)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 56)
ALL POSITIONS RIBONUCLEOTIDE EXCEPT POSITIONS (N N)

B

SENSE STRAND (SEQ ID NO 57)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-OM EXCEPT POSITIONS (N N)

5'- N N N N N N N N N N N N N N N N N N N (N_SN) -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 58)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

C

SENSE STRAND (SEQ ID NO 59)
ALL PYRIMIDINES = 2'-O-ME OR 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 60)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

D

SENSE STRAND (SEQ ID NO 61)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 58)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

E

SENSE STRAND (SEQ ID NO 62)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N)

5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 58)
ALL PYRIMIDINES = 2'-FLUORO AND ALL PURINES = 2'-O-ME EXCEPT POSITIONS (N N)

F

SENSE STRAND (SEQ ID NO 61)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

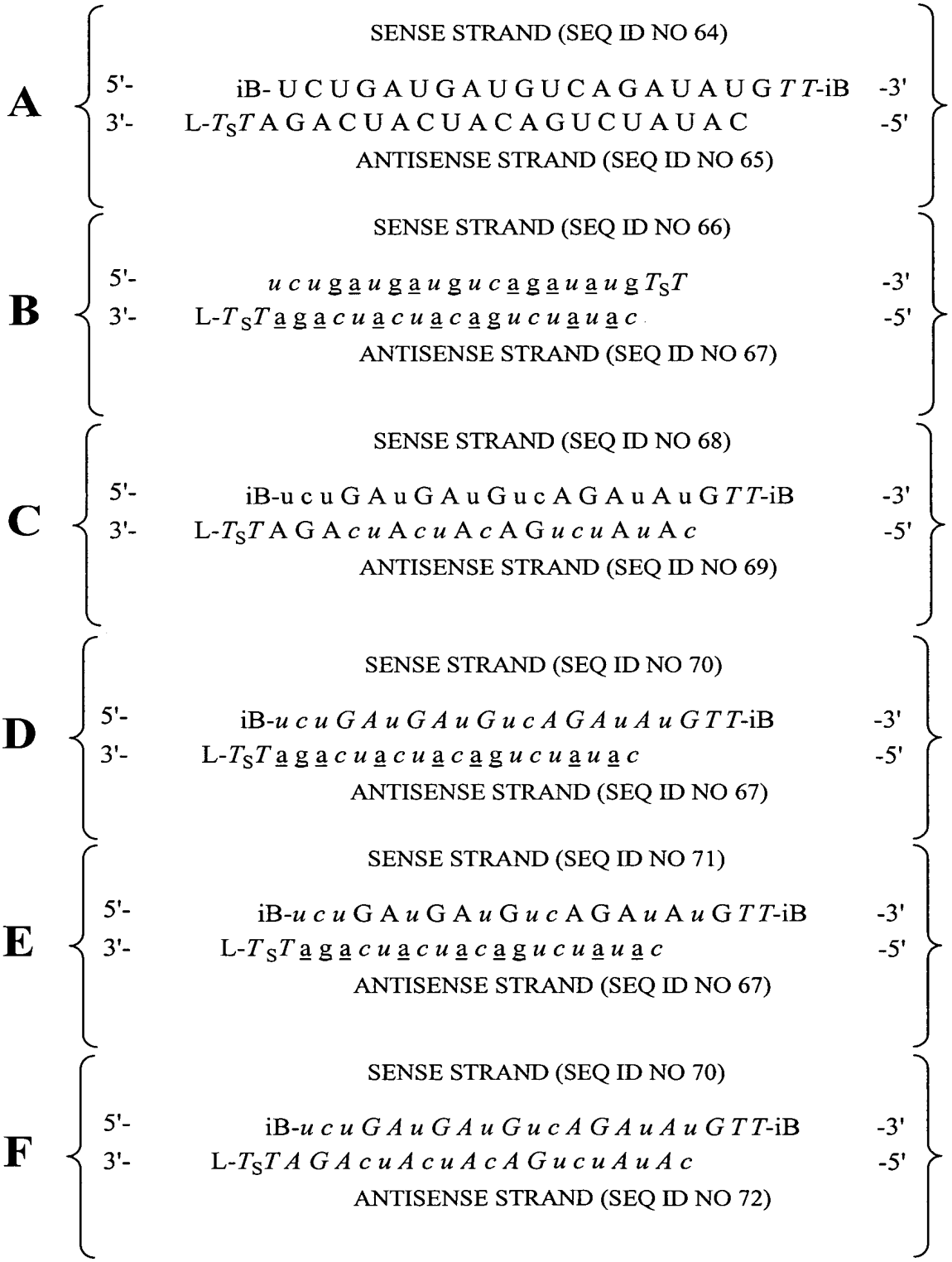
5'- B-N N N N N N N N N N N N N N N N N N N (N N)-B -3'

3'- L-(N_SN) N N N N N N N N N N N N N N N N N N N -5'

ANTISENSE STRAND (SEQ ID NO 63)
ALL PYRIMIDINES = 2'-FLUORO EXCEPT POSITIONS (N N) AND ALL PURINES = 2'-DEOXY

POSITIONS (NN) CAN COMPRISE ANY NUCLEOTIDE, SUCH AS DEOXYNUCLEOTIDES (eg. THYMIDINE) OR UNIVERSAL BASES
B = ABASIC, INVERTED ABASIC, INVERTED NUCLEOTIDE OR OTHER TERMINAL CAP THAT IS OPTIONALLY PRESENT
L = GLYCERYL or B THAT IS OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR PHOSPHORODITHIOATE that is optionally absent

Figure 5



lower case = 2'-O-Methyl or 2'-deoxy-2'-fluoro

italic lower case = 2'-deoxy-2'-fluoro

underline = 2'-O-methyl

ITALIC UPPER CASE = DEOXY
iB = INVERTED DEOXYABASIC
L = GLYCERYL MOIETY or iB OPTIONALLY PRESENT
S = PHOSPHOROTHIOATE OR
PHOSPHORODITHIOATE OPTIONALLY PRESENT

Figure 6

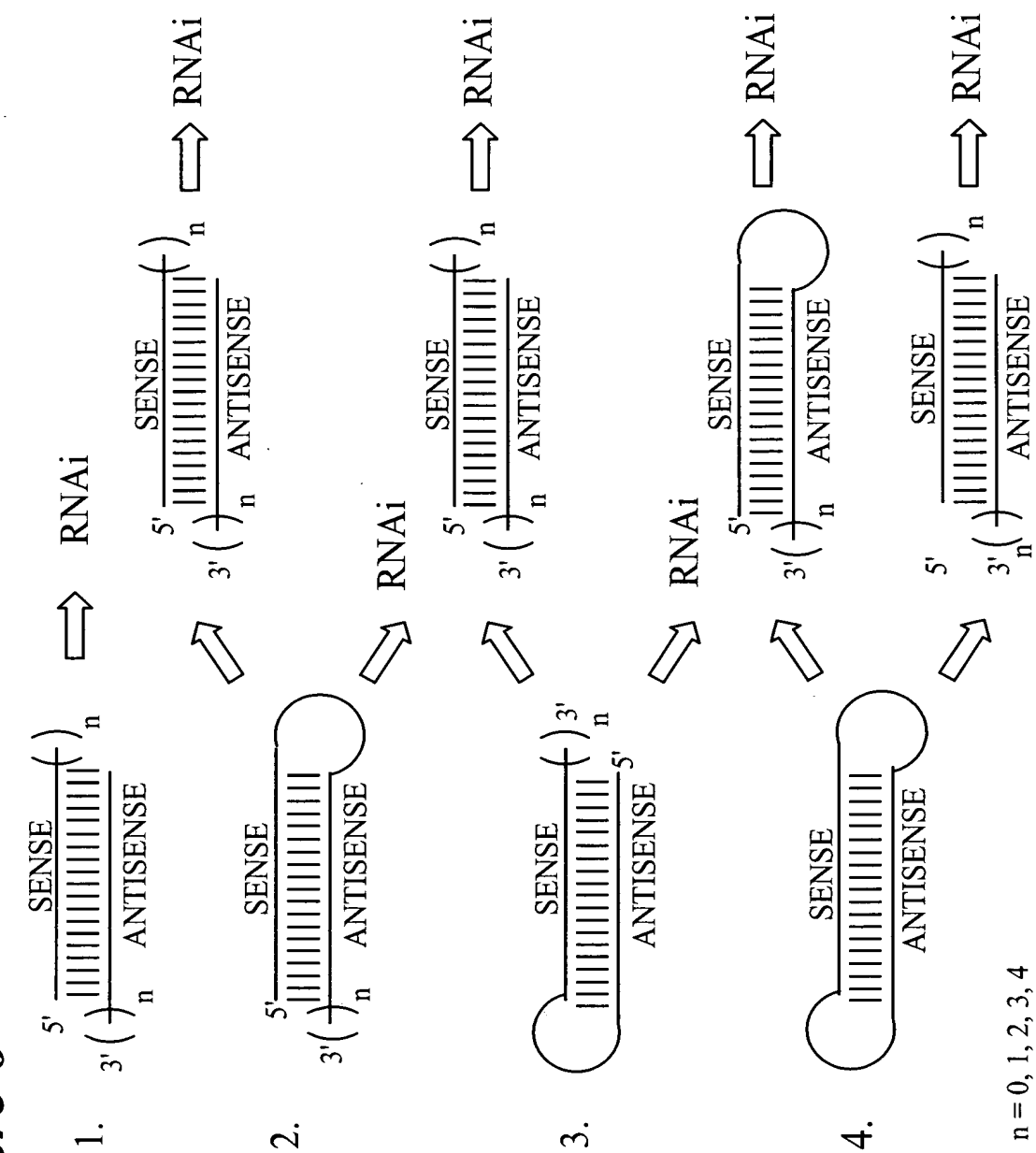
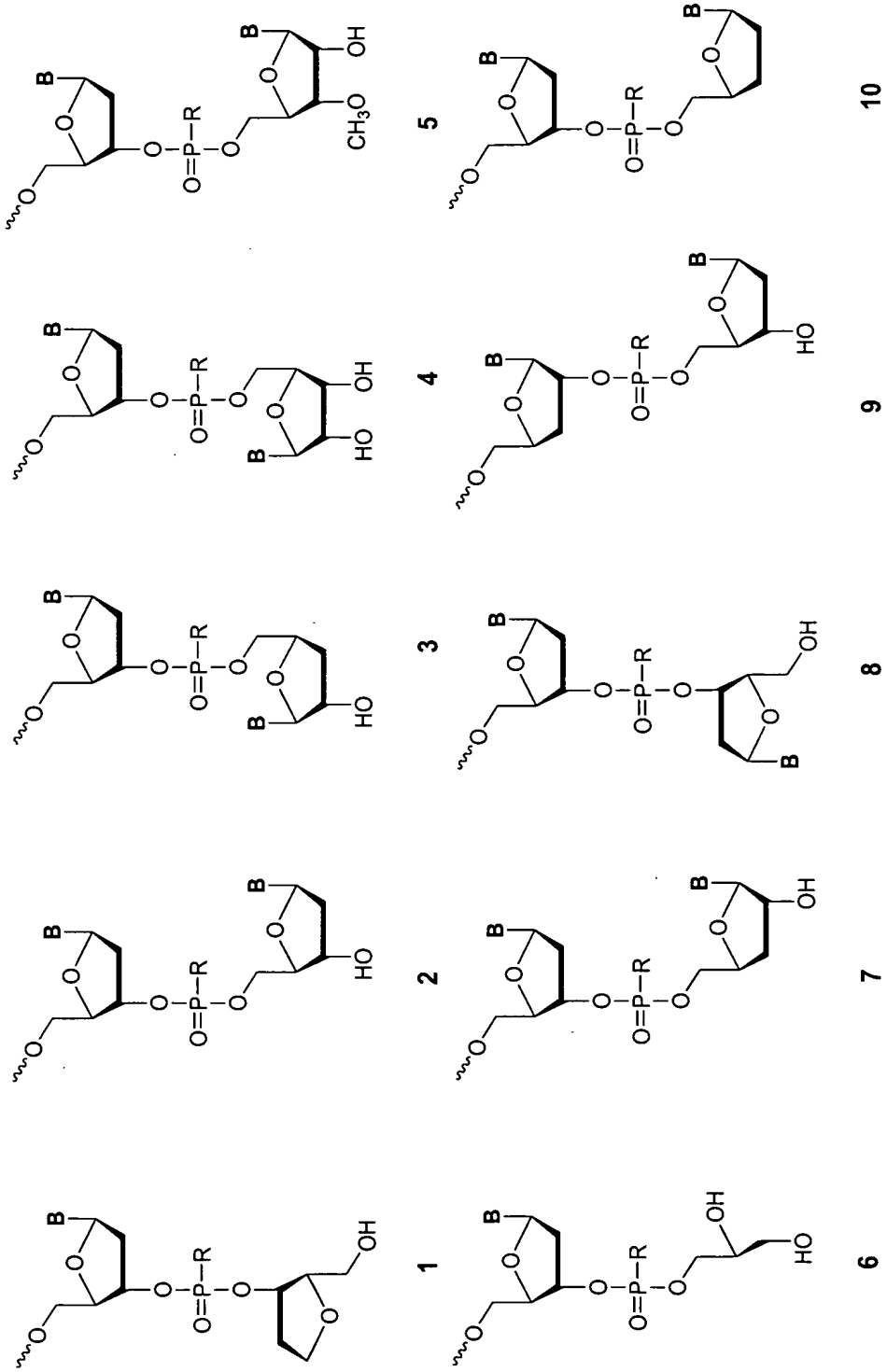
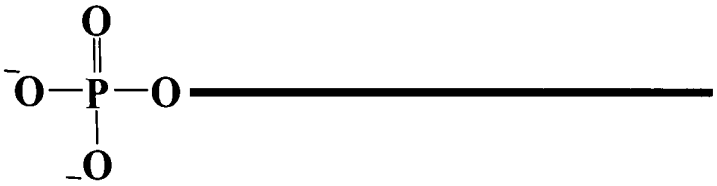


Figure 7



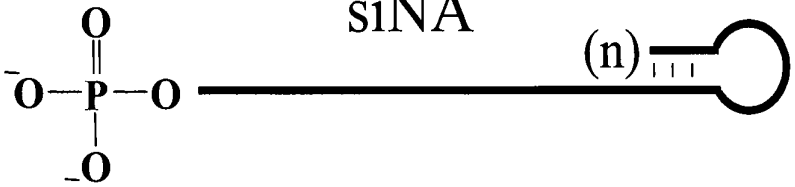
R = O, S, N, alkyl, substituted alkyl, O-alkyl, S-alkyl, alkaryl, or aralkyl
B = Independently any nucleotide base, either naturally occurring or chemically modified, or optionally H (abasic).

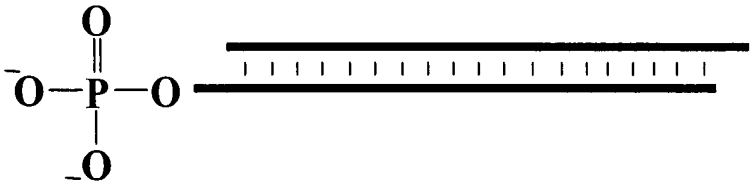
Figure 8: Phosphorylated siNA constructs



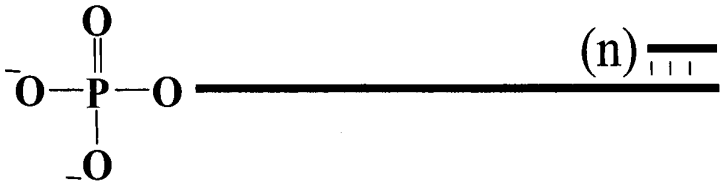
Phosphates can be modified as described herein

Asymmetric hairpin siNA





Asymmetric duplex siNA



(n) = number of base pairs (e.g. 3-18 bp)

Figure 9: 5'-phosphate modifications

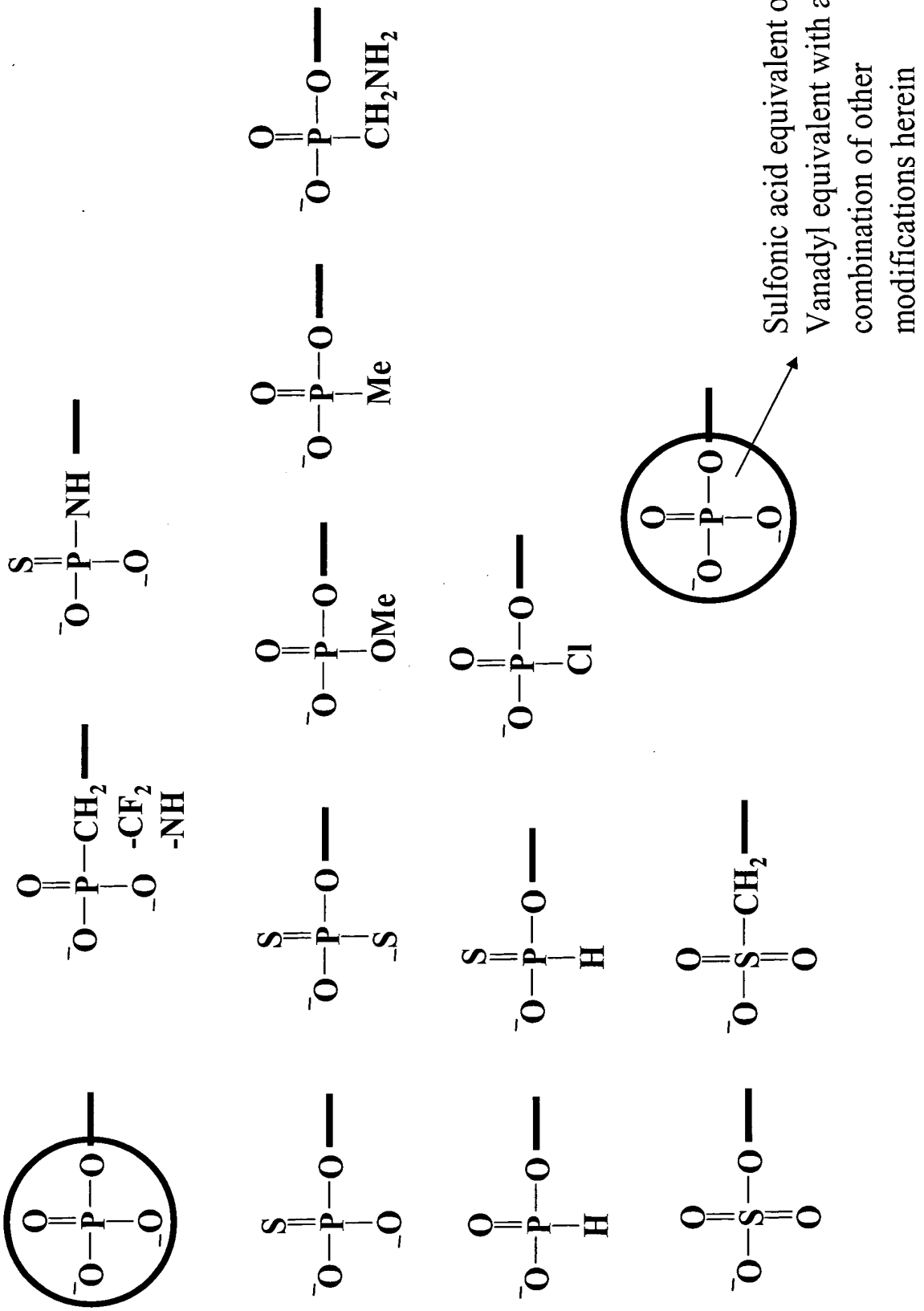


Figure 10A: Duplex forming oligonucleotide constructs that utilize palindrome or repeat sequences

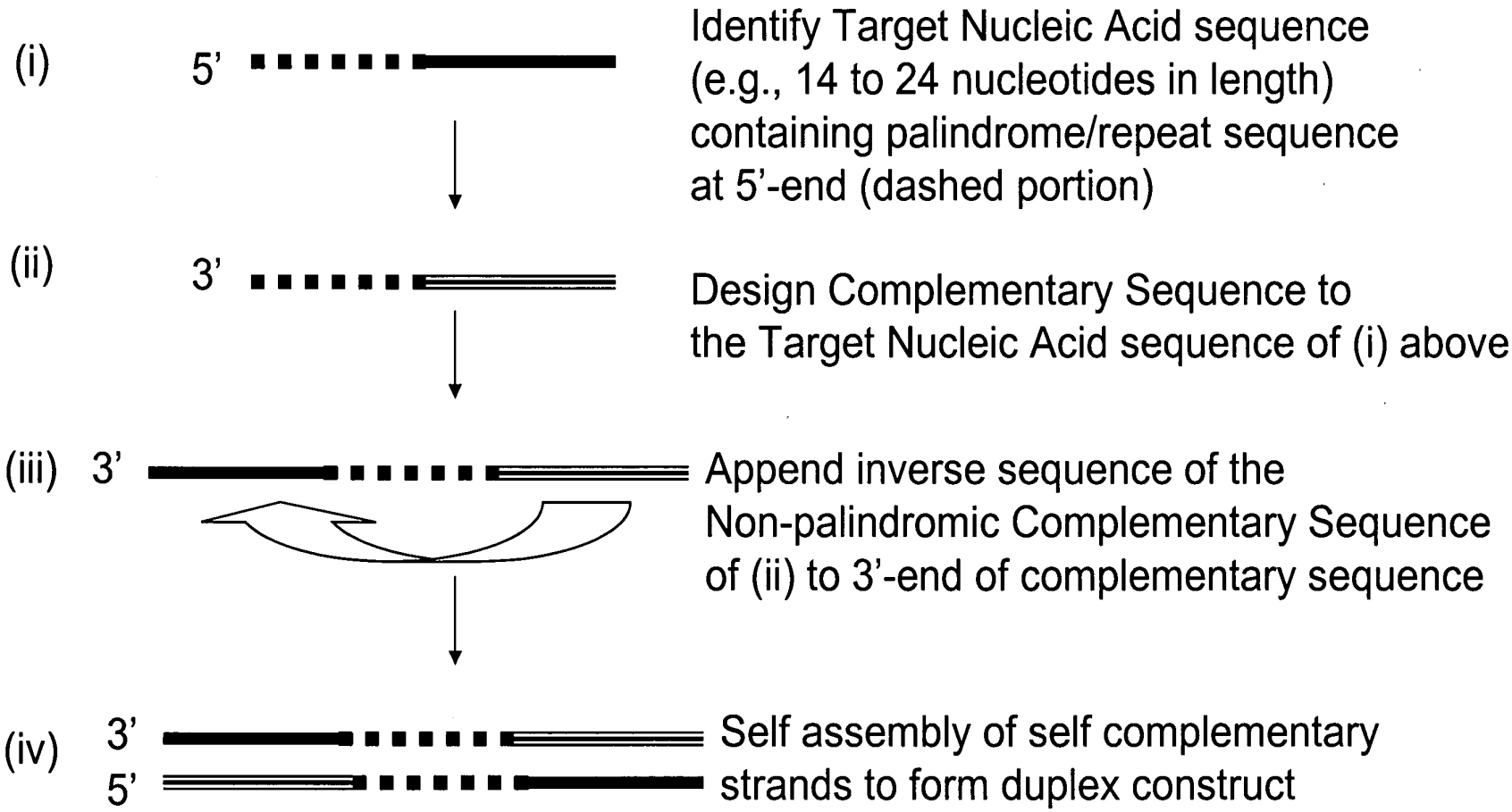
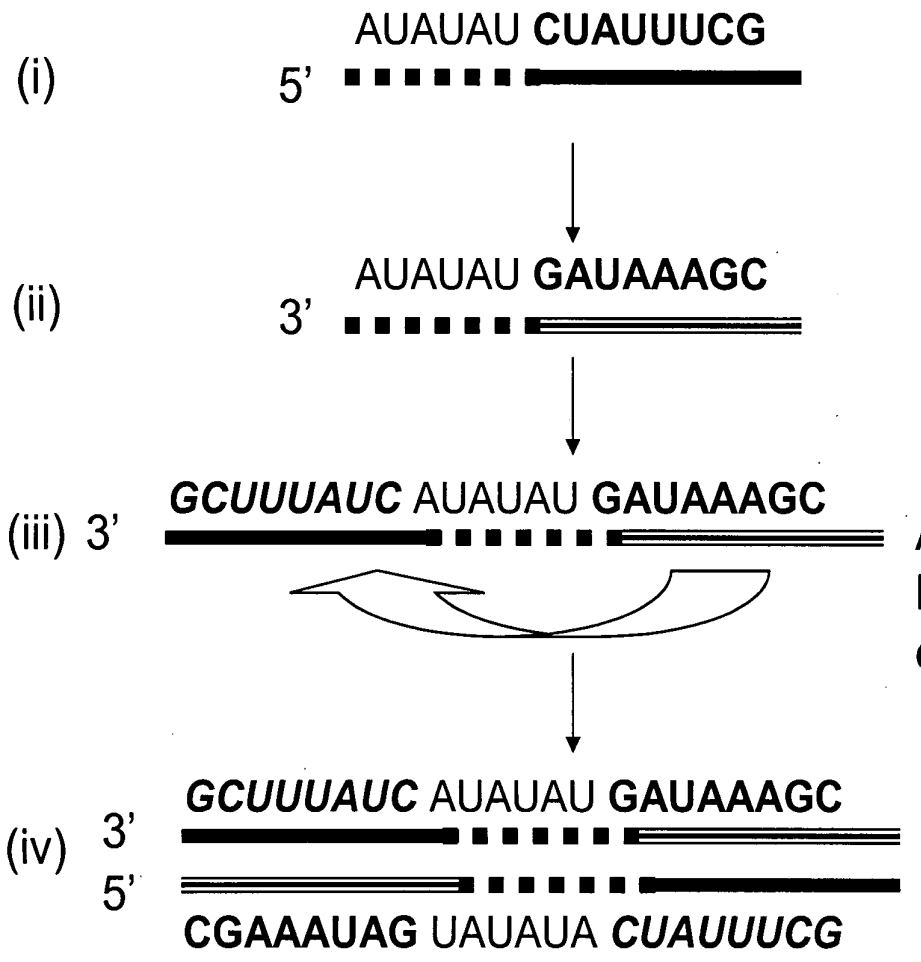


Figure 10B: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length) containing palindrome/repeat sequence at 5'-end (dashed portion)

Design Complementary Sequence to the Target Nucleic Acid sequence of (i) above

Append inverse sequence of the Non-palindromic Complementary Sequence of (ii) to 3'-end of complementary sequence

Self assembly of self complementary strands to form duplex construct (blunt ends)

Figure 10C: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly

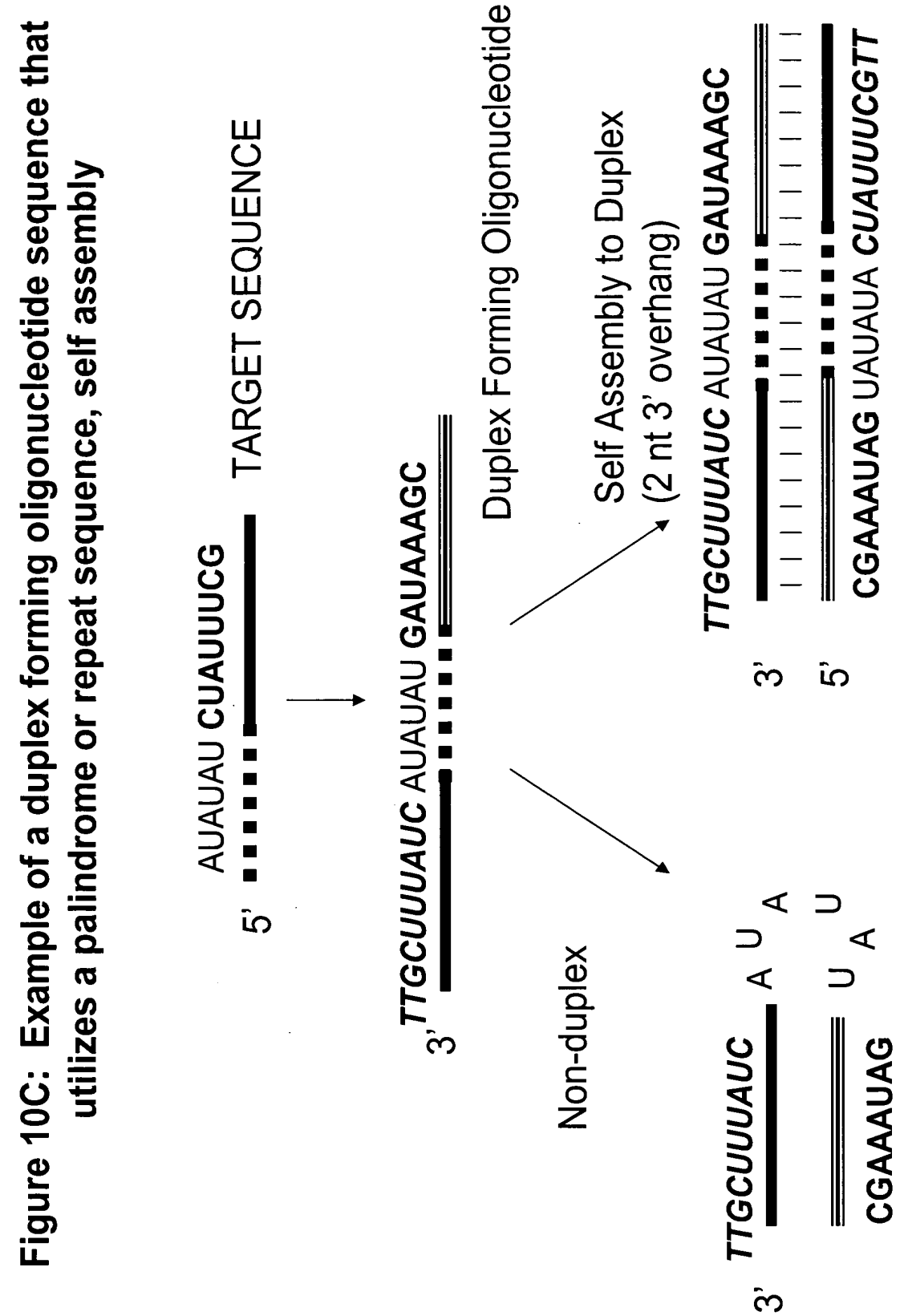


Figure 10D: Example of a duplex forming oligonucleotide sequence that utilizes a palindrome or repeat sequence, self assembly and inhibition of Target Sequence Expression

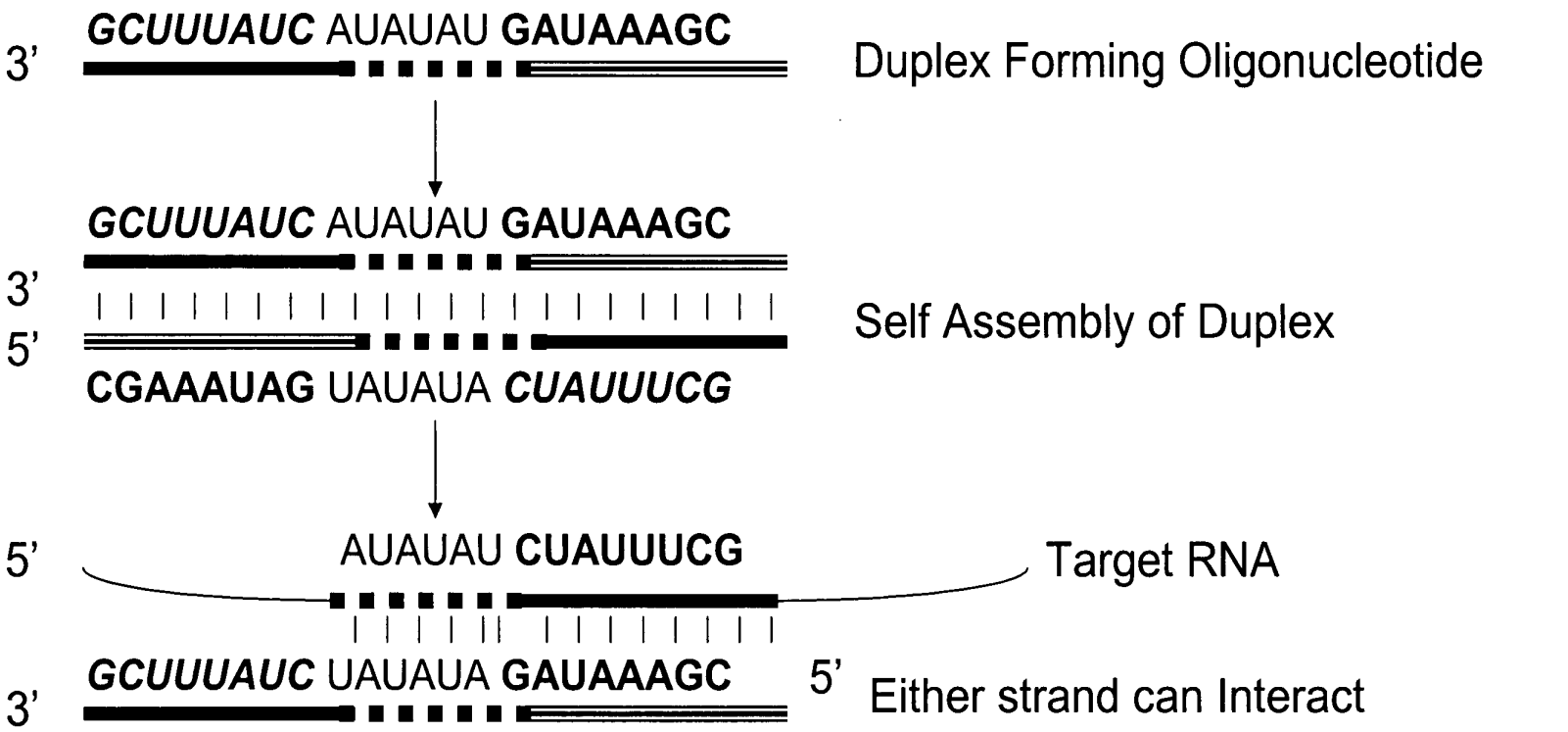
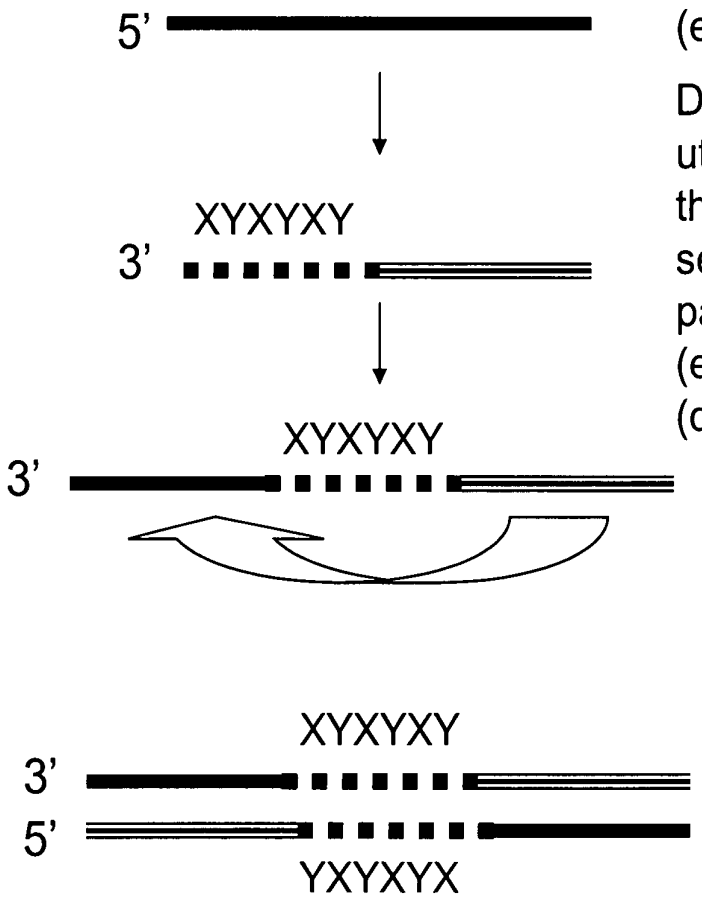


Figure 11: Duplex forming oligonucleotide constructs that utilize artificial palindrome or repeat sequences



Identify Target Nucleic Acid sequence (e.g., 14 to 24 nucleotides in length)

Design Complementary Sequence and utilize modified nucleotides (shown as X, Y) that interact with a portion of the target sequence and result in the formation of a palindrome/repeat sequence (e.g., 2 to 12 nucleotides) at 3'-end (dashed portion)

Append inverse sequence of Complementary region to 3'-end of palindrome/repeat sequence

Hybridize self complementary strands to form duplex siNA construct

Figure 12: Examples of double stranded multifunctional siNA constructs with distinct complementary regions

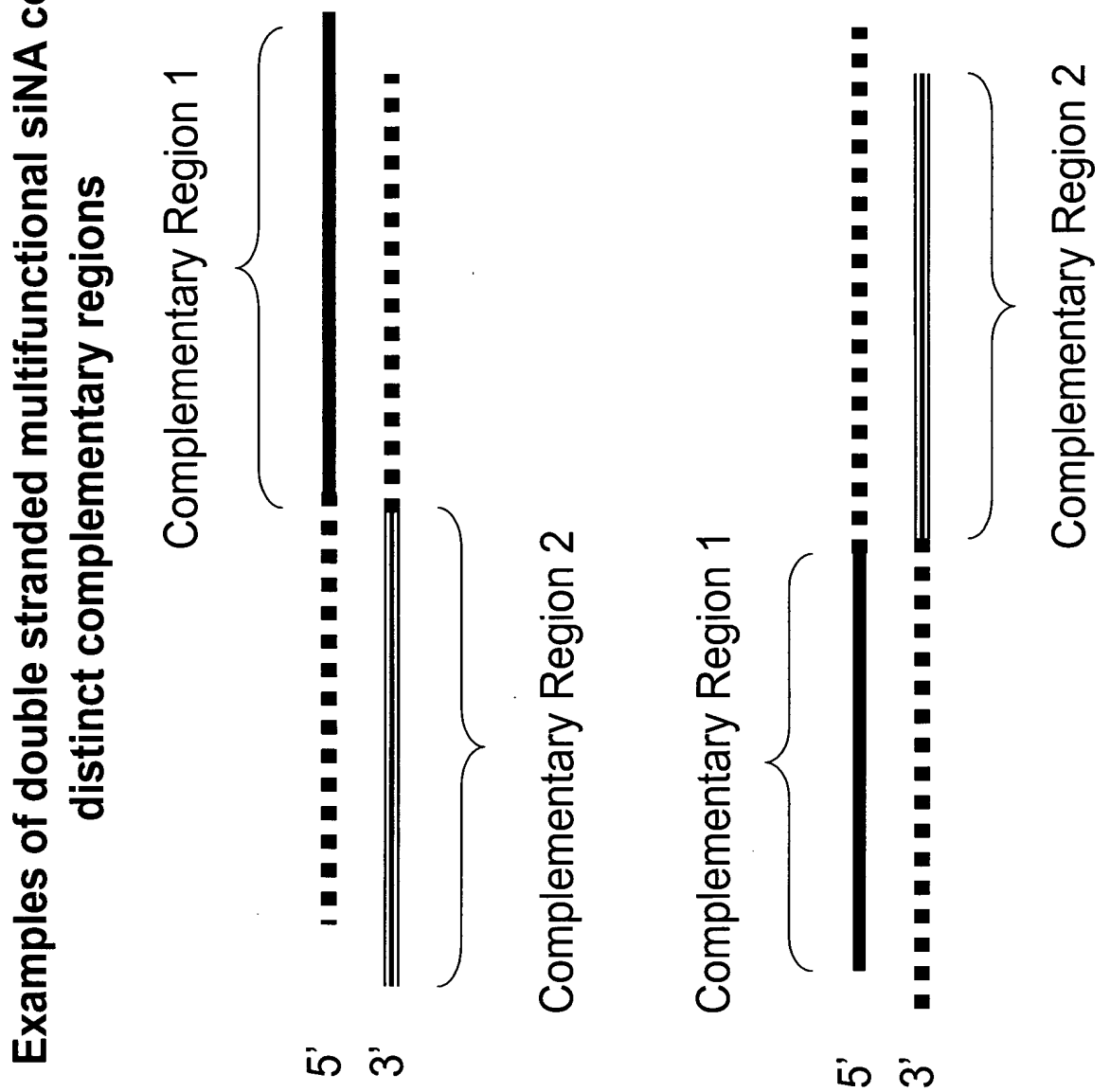


Figure 13: Examples of hairpin multifunctional siNA constructs with distinct complementary regions

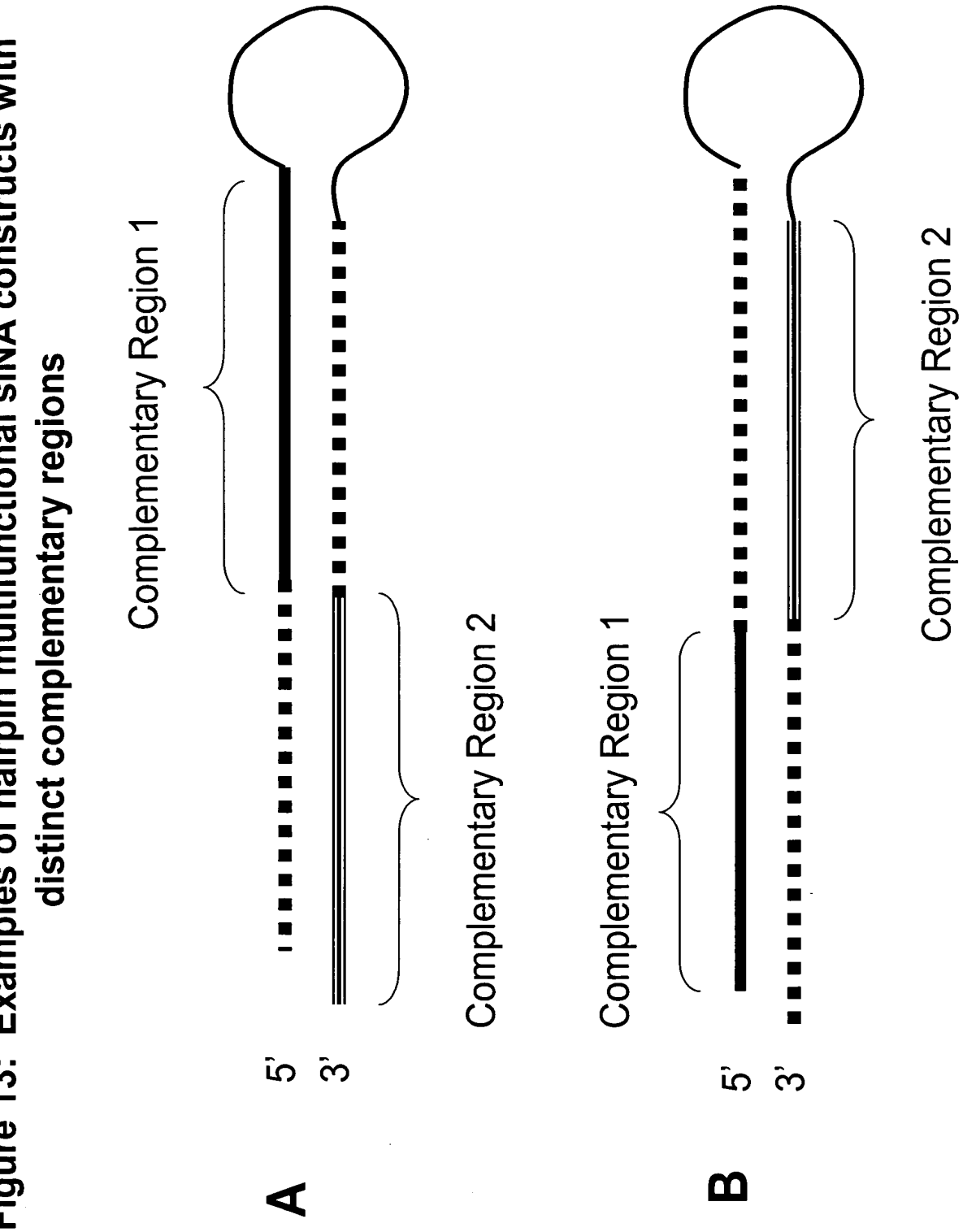


Figure 14: Examples of double stranded multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region

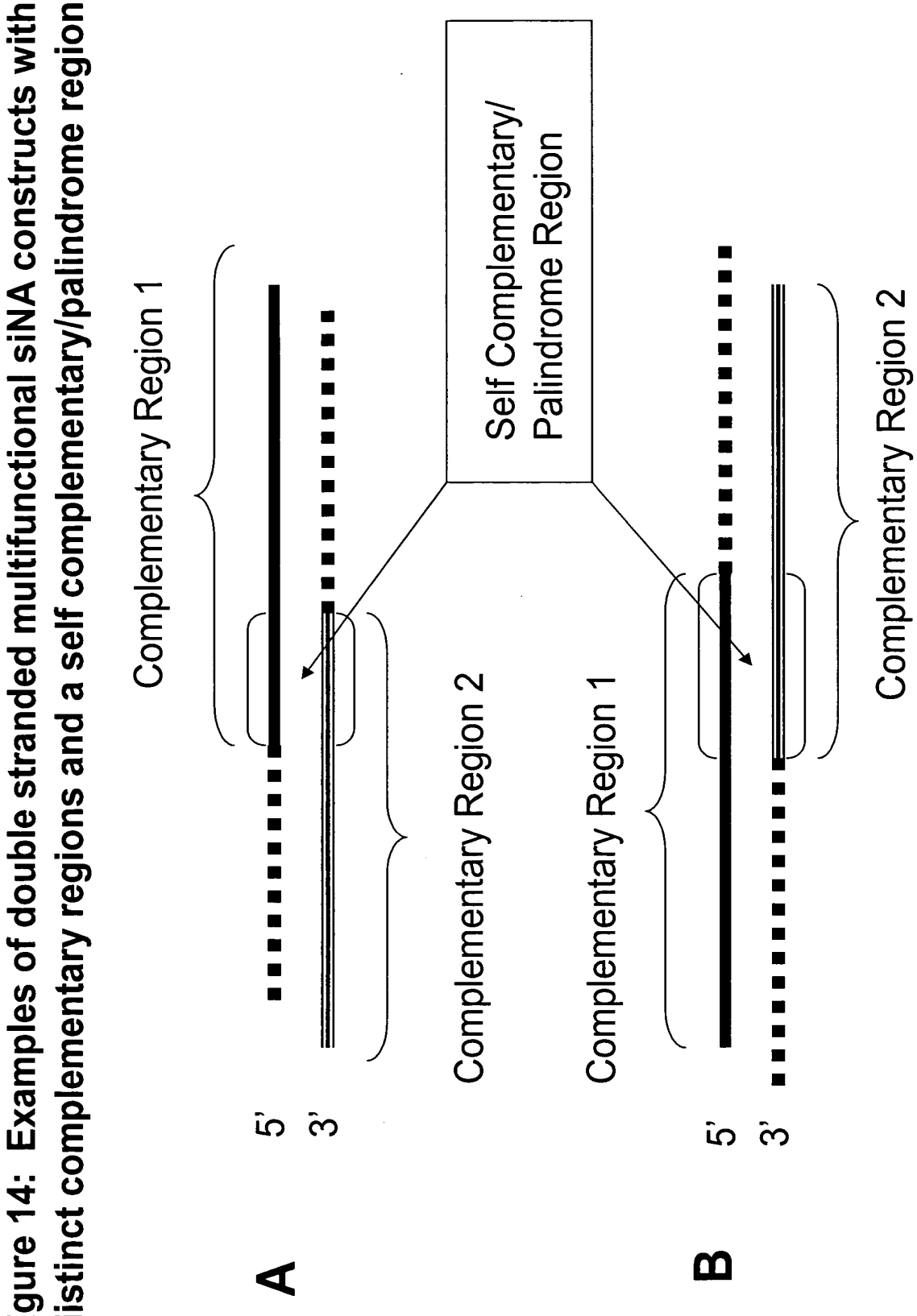
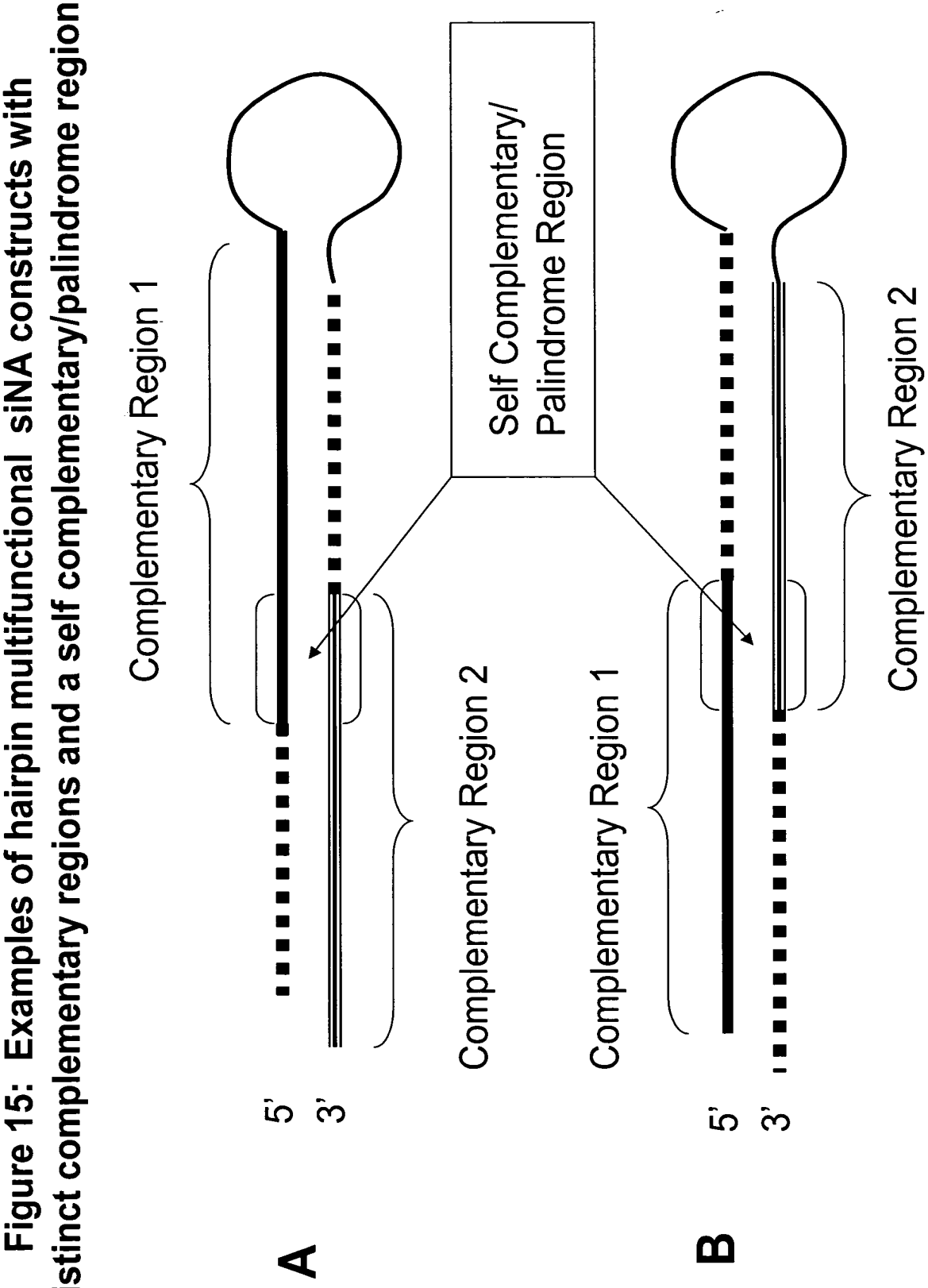


Figure 15: Examples of hairpin multifunctional siNA constructs with distinct complementary regions and a self complementary/palindrome region



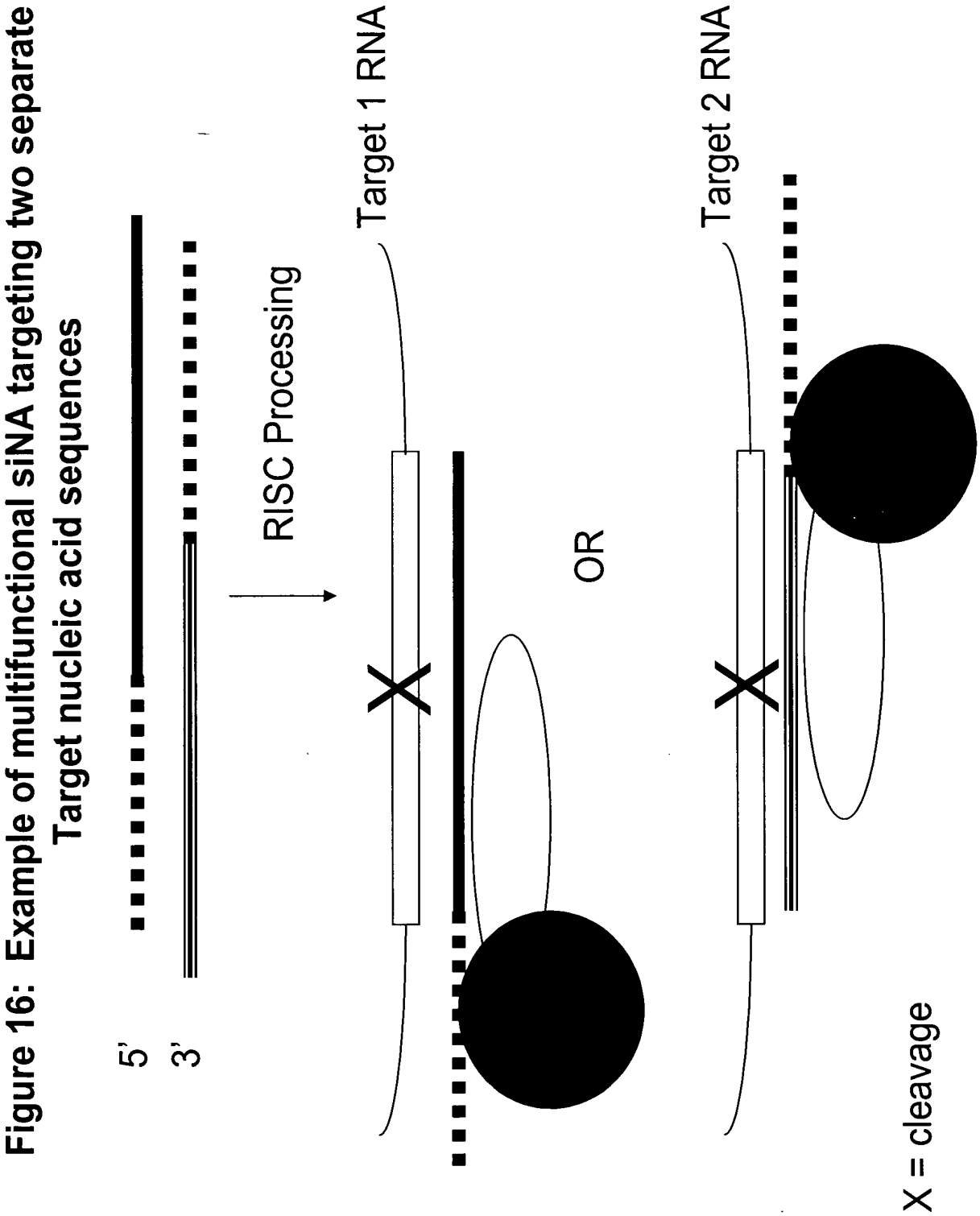


Figure 16: Example of multifunctional siNA targeting two separate

Figure 17: Example of multifunctional siNA targeting two regions within the same target nucleic acid sequence

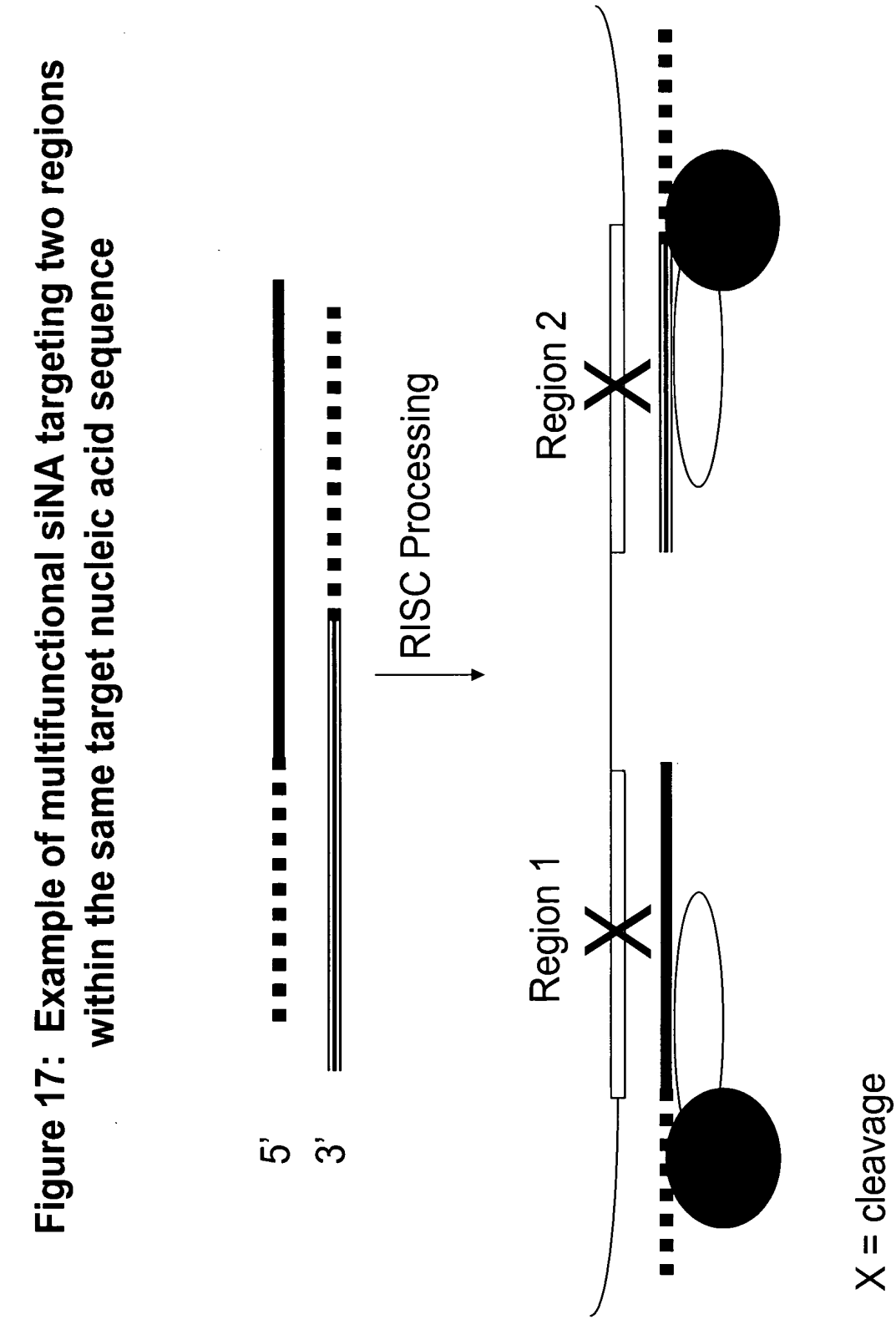
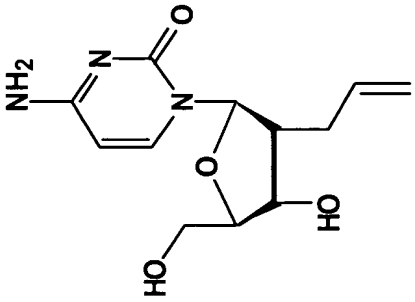
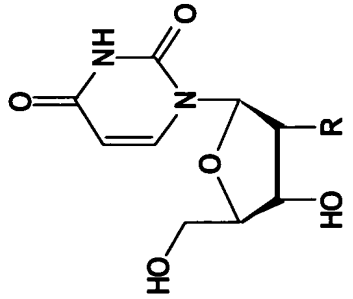


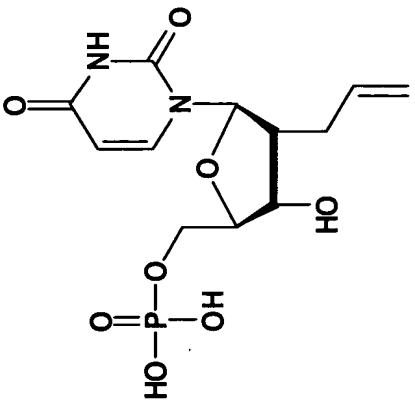
Figure 18



7



2. R = NH_2
3. R = OCH_3
4. R = H
5. R = OCH_2-CH=CH_2
6. R = $CH_2CH_2CH_3$
8. R = F



1

Figure 19

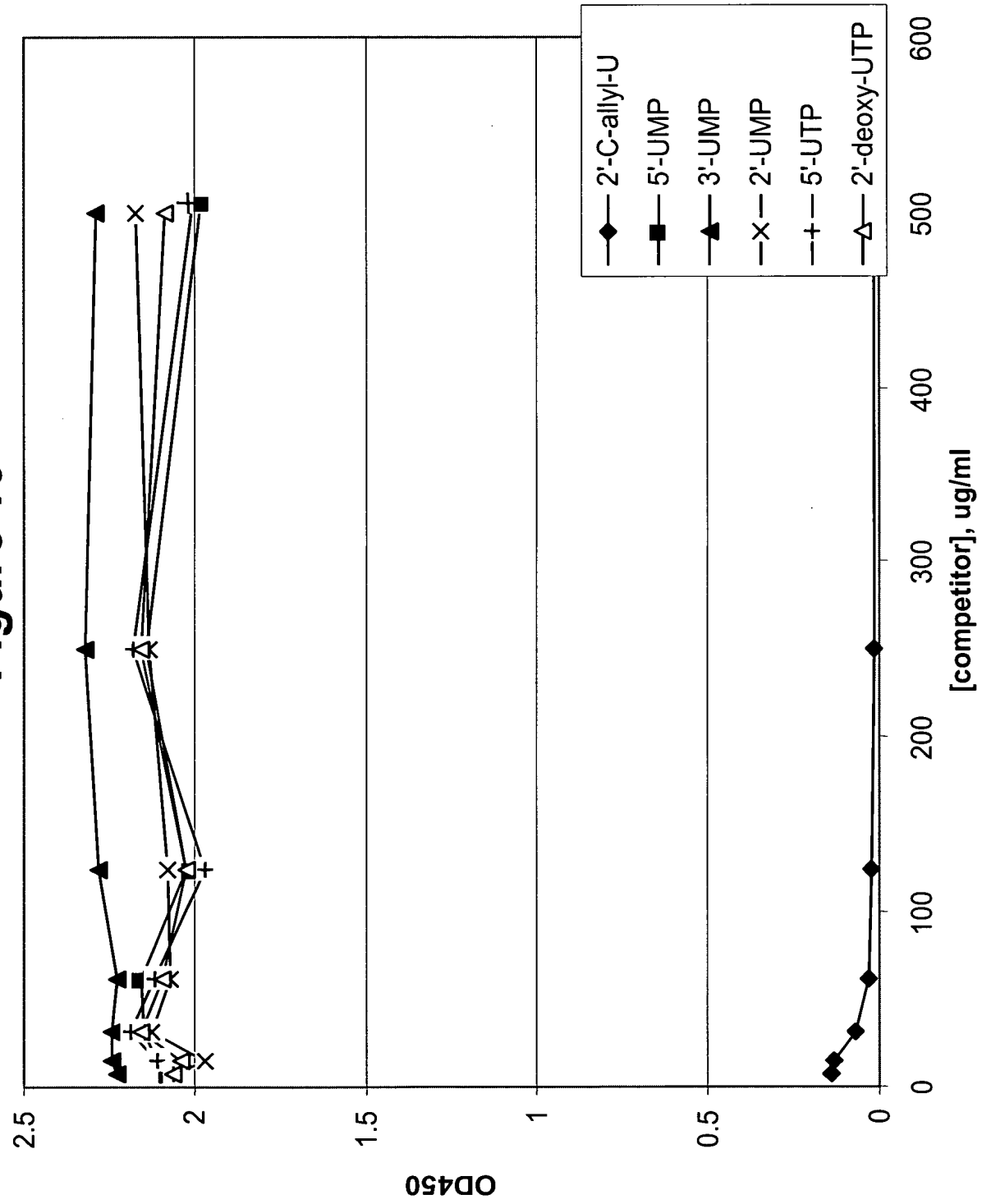
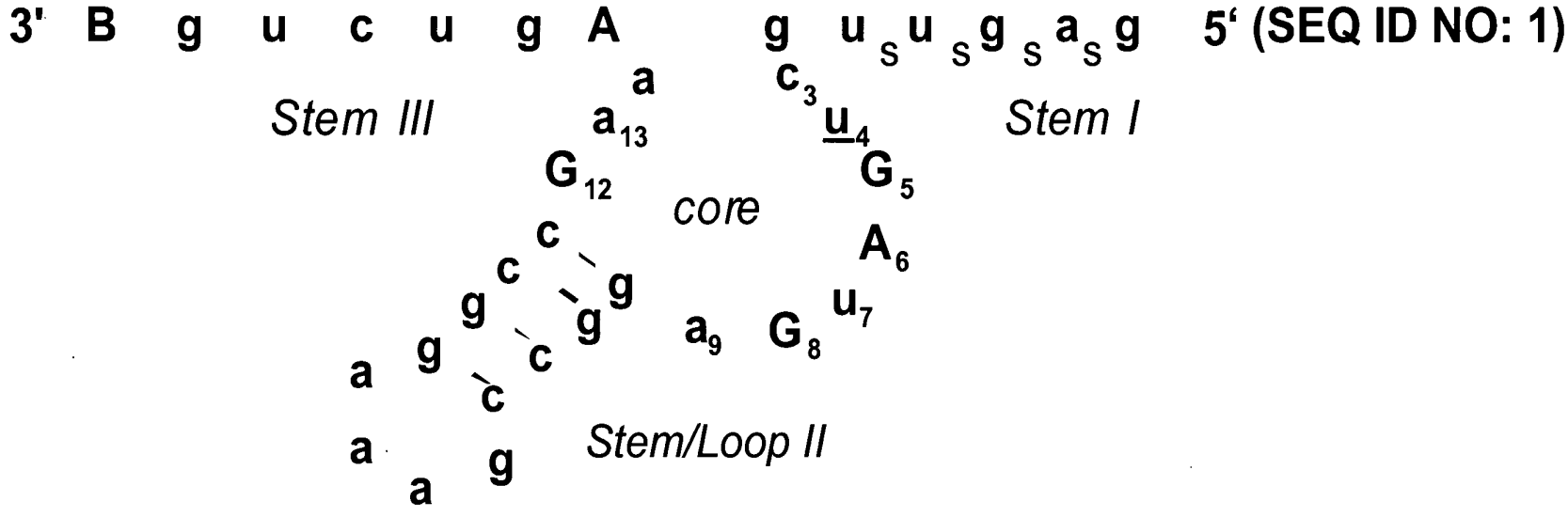

OD450
2.5
2
1.5
1
0.5
0
0
100
200
300
400
500
600
[competitor], ug/ml
2'-C-allyl-U
5'-UMP
3'-UMP
2'-UMP
5'-UTP
2'-deoxy-UTP

Figure 20



ANGIOZYME™

Uppercase: ribonucleotide

Lowercase: 2'-O-Me nucleotide

U: **2'-deoxy-2'-C-allyl Uridine**

B: inverted 2'-deoxyribose abasic

S: phosphorothioate linkage

Figure 21

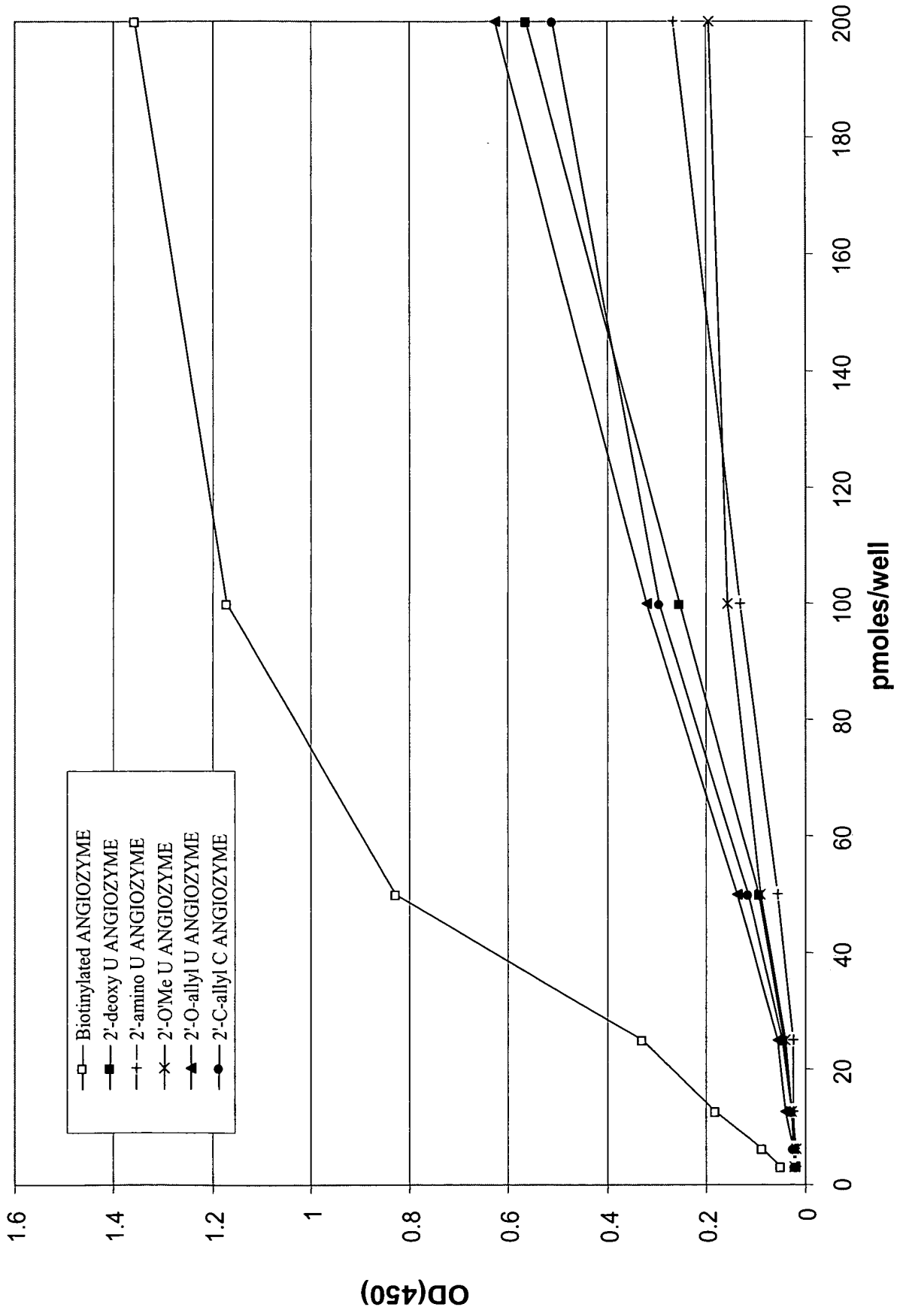

OD(450)
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
pmoles/well
0
20
40
60
80
100
120
140
160
180
200
Biotinylated ANGIOZYME
2'-deoxy U ANGIOZYME
2'-amino U ANGIOZYME
2'-OMe U ANGIOZYME
2'-O-allyl U ANGIOZYME
2'-C-allyl C ANGIOZYME

Figure 22

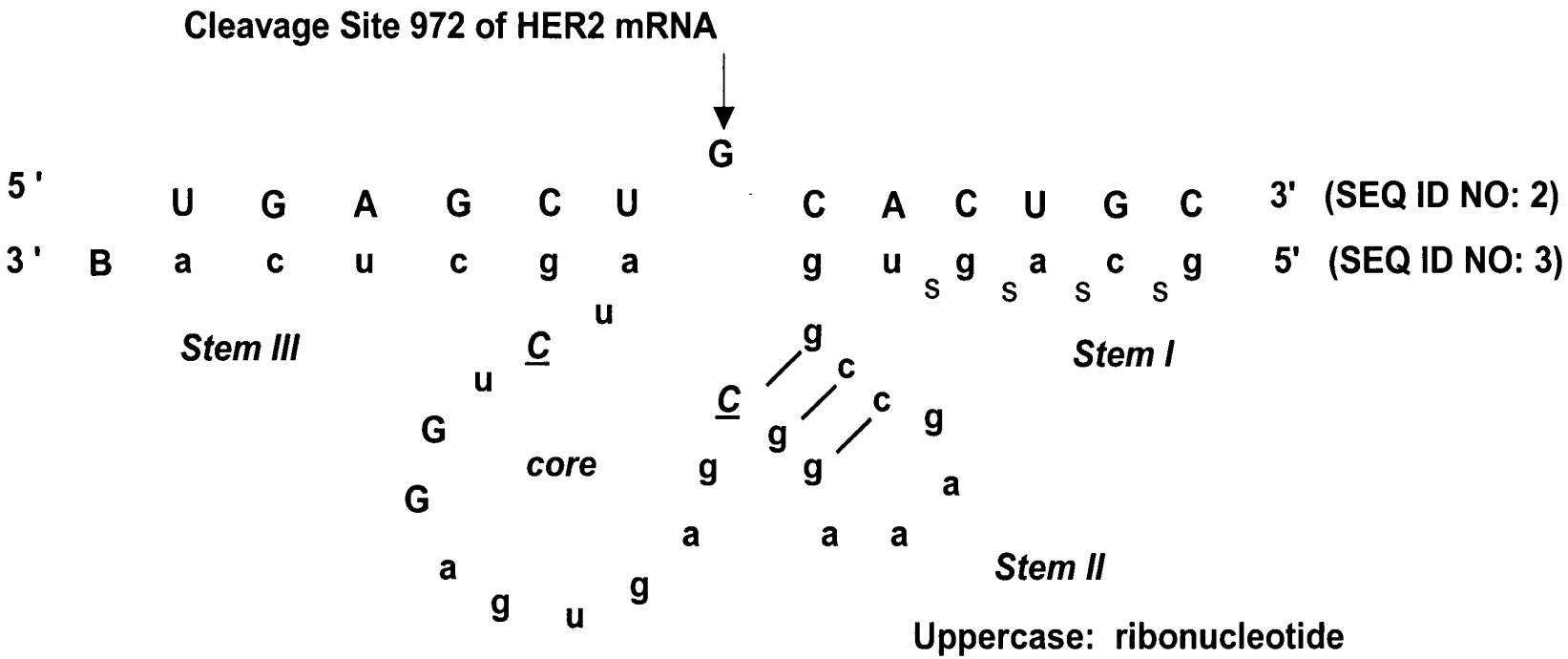

Cleavage Site 972 of HER2 mRNA
G
5 ' U G A G C U C A C U G C 3' (SEQ ID NO: 2)
3 ' B a c u c g a g u g a c g 5' (SEQ ID NO: 3)
s s s s
u
Stem III
C
g
c
u
C
c
G
g
core
g
g
a
G
a
a a
a
g
g
u
Stem I
Stem II
RPI.19293
HERZYME™
Uppercase: ribonucleotide
Lowercase: 2'-O-Me nucleotide
C: 2'-deoxy-2'-amino Cytidine
B: inverted 2'-deoxyribose abasic
S: phosphorothioate linkage

Figure 23

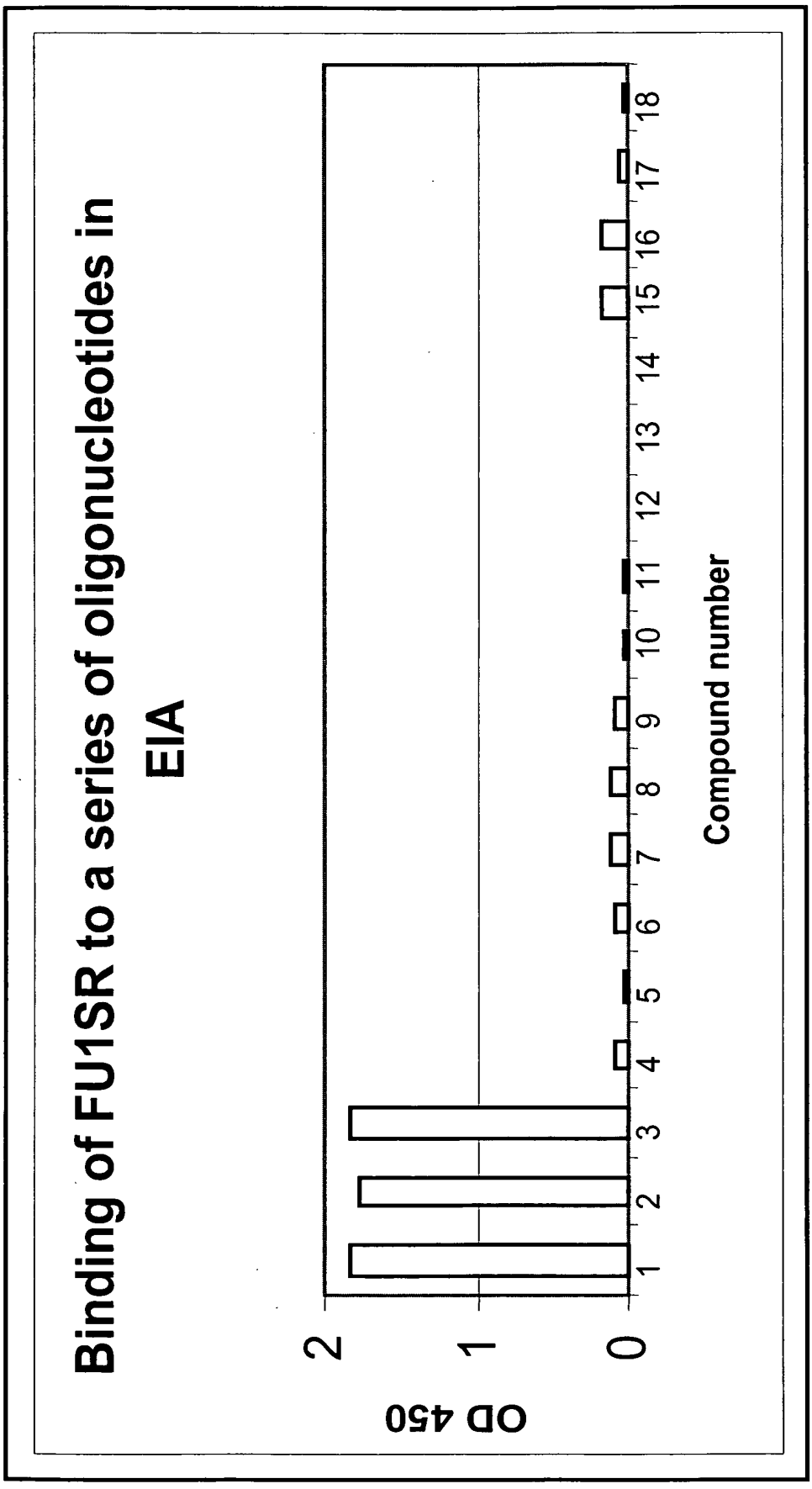
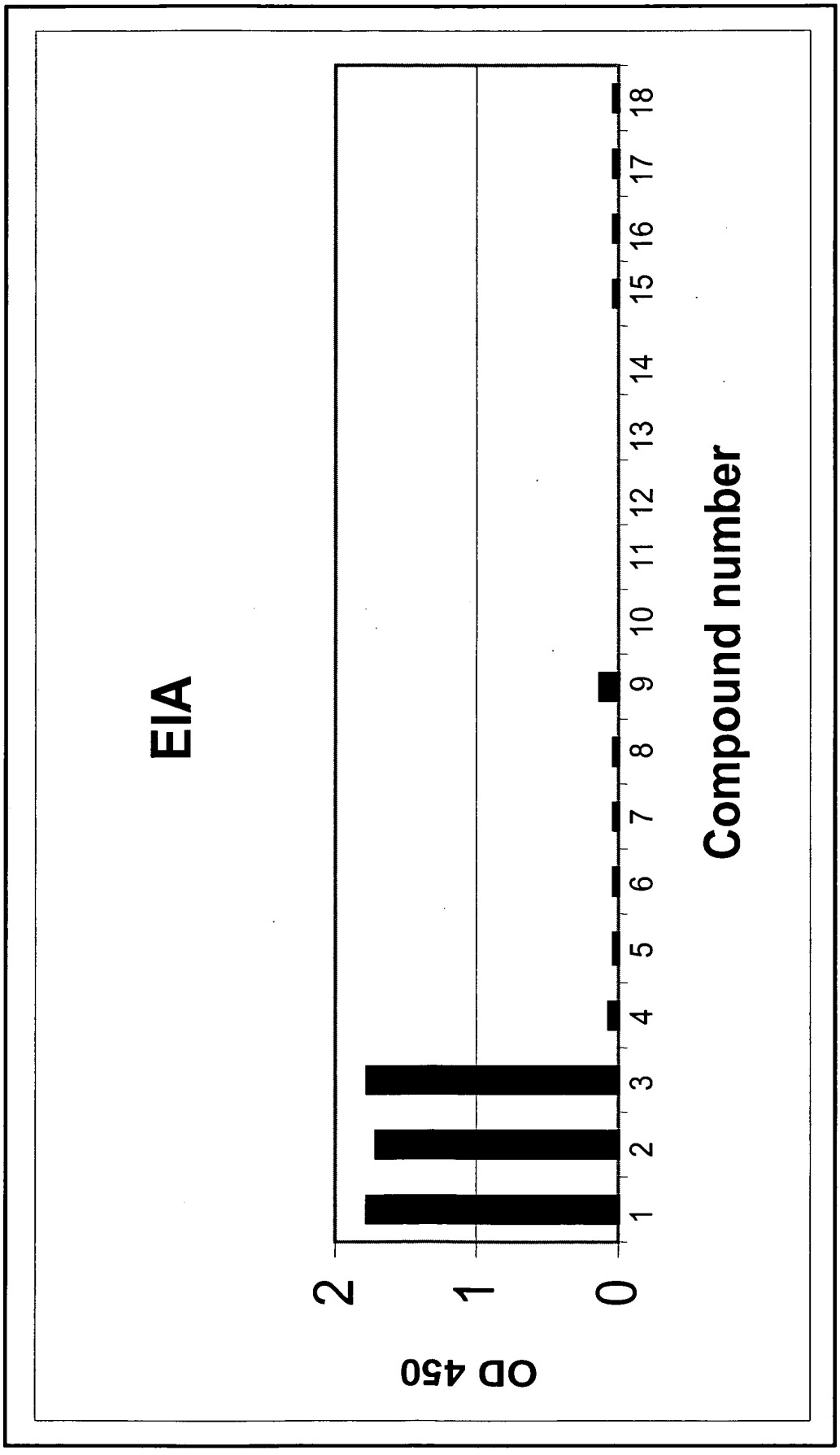

Binding of FU1SR to a series of oligonucleotides in EIA
OD 450
0
1
2
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Compound number

Figure 24


EIA
OD 450
2
1
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
Compound number